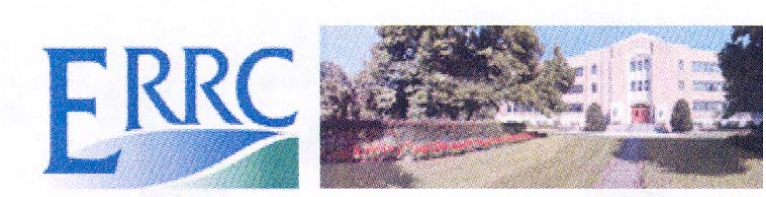

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Gamma Irradiation of Fine-Emulsion Sausage Containing Sodium Diacetate†

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ABSTRACT

Listeria monocytogenes, a psychrotrophic foodborne pathogen, is a frequent postprocess contaminant of ready-to-eat (RTE) meat products, including frankfurters and bologna. Ionizing radiation can eliminate *L. monocytogenes* from RTE meats. Sodium diacetate (SDA) incorporated into fine-emulsion sausages inhibits the growth of *L. monocytogenes.* Irradiation of *L. monocytogenes* suspended in SDA solutions resulted in synergistic reductions of the microorganism. *L. monocytogenes* populations were reduced by >9 $\log_{10}$ units at a radiation dose of 1.5 kGy when suspended in 0.125% SDA solution. In contrast, the D_{10}-values (the ionizing radiation doses required to reduce the population by 90%) were 0.58, 0.59, 0.57, and 0.53 kGy for *L. monocytogenes* populations suspended in emulsions containing 0, 0.125, 0.25, and 0.5% SDA, respectively. The D_{10}-values for *L. monocytogenes* surface inoculated onto frankfurters dipped in 0, 0.125, 0.25, and 0.5% SDA solutions were 0.58, 0.53, 0.54, and 0.52 kGy, respectively. Postirradiation growth of *L. monocytogenes* suspended in beef bologna emulsion at 9°C was dependent on SDA concentration and ionizing radiation dose. Very small, but statistically significant, changes in bologna redness, lipid oxidation, and shear force were observed for the beef bologna emulsion with the highest SDA concentration (0.5%) and irradiation dose (3.0 kGy). SDA can inhibit the proliferation of *L. monocytogenes* surviving the irradiation process with minimal impact on fine-emulsion sausage color, lipid oxidation, and firmness when used within regulatory limits.

Listeria monocytogenes is a frequent postprocess contaminant of ready-to-eat (RTE) meat products, and a number of foodborne illness outbreaks have been attributed to this microorganism *(2, 4, 5, 13). L. monocytogenes* is capable of growth at refrigerated temperatures and in high-salt environments, which allows it to proliferate during long-term storage *(17).* Because of the high mortality rate associated with listeriosis, *L. monocytogenes* is given zero tolerance in RTE meat products in the United States *(9, 25).*

Ionizing radiation is a safe and effective method for the elimination of *L. monocytogenes* from RTE meats *(6, 18–22).* A petition has been filed with the U.S. Food and Drug Administration to allow ionizing radiation pasteurization of RTE foods, including RTE meat products such as bologna and frankfurters *(12).* Potassium lactate and sodium diacetate alone or in combination can inhibit the growth of *L. monocytogenes* on RTE meats during long-term refrigerated storage *(1, 3, 8, 14–16, 23).* Although both lactate and diacetate are approved for use in RTE meat products *(26),* little, if any, data exist on the effects of ionizing radiation and lactate or diacetate on the viability and growth potential of *L. monocytogenes.*

In this study, the following questions were addressed. (i) What are the effects of ionizing radiation and SDA on the radiation resistance of *L. monocytogenes* inoculated into SDA solutions, surface inoculated onto frankfurters dipped in SDA solutions, and suspended in cooked beef bologna emulsions containing various levels of SDA? (ii) What are the effects of ionizing radiation and SDA on the postirradiation growth of *L. monocytogenes*? (iii) What are the effects of SDA and ionizing radiation on cooked bologna emulsion lipid oxidation, color, and firmness?

MATERIALS AND METHODS

Sausage manufacture. Ground beef (15% fat) was emulsified in a Hobart Model HCM40 Cutter-Mixer. Cure ingredients and additives (wt/wt per kg of meat) included 3% sodium chloride, 3% dextrose, 0.5% sodium tripolyphosphate, 0.05% sodium erythorbate, 0.02% sodium nitrite, and 20% deionized water. SDA was added as needed to obtain the required final concentrations and was the only experimental variable other than radiation dose. Spices were not used in order to limit the number of experimental variables. Emulsions that did not contain SDA were stuffed into 22-mm cellulose (Dewied International Inc., Santa Fe, N.M.) casings to produce frankfurters. Emulsions with no diacetate and emulsions with diacetate (at 0.125 to 0.5%) were stuffed into 10-cm fibrous casings (Dewied International) to produce bologna. The sausages were then cooked in a Koch Model KL-50 Smokehouse (Koch Inc., Kansas City, Mo.) to an internal product temperature of 73°C. The dry bulb setting was 90°C, and the wet bulb setting was 63°C, for a relative humidity of approximately 47%.

After the desired internal temperature was reached, the sausages were immediately chilled in a sterile cold water bath, and the casings were removed in an aseptic manner. The sausages were then sectioned and vacuum packaged to 0.26 mm Hg (ca. 34.66 Pa) with a Multi-Vac A300 Vacuum Packager (Multi-Vac, Kansas City, Mo.), overpacked in gas- and moisture-impermeable Mil-B-131-H foil bags (Bell Fibre Products Corp., Columbus, Ga.), and

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† Mention of a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

stored at 0 to 2°C until they were ready for use. Cooked-emulsion pHs were 6.7, 6.6, 6.3, and 5.9 for bologna containing 0, 0.125, 0.25, and 0.5% SDA, respectively. Background microfloras consisted of <1 CFU/g or <1 CFU/cm^2 bologna for all formulations.

Strains. Four *L. monocytogenes* strains (H7595, H7762, H7969, and H7962) isolated from RTE meats were obtained from the Centers for Disease Control and Prevention (Atlanta, Ga.). The strains were propagated on Palcam agar (Difco Laboratories, Sparks, Md.) at 37°C and maintained at 0 to 2°C until they were ready for use. *Listeria* were confirmed by Gram staining followed by analysis with Gram Positive Identification (GPI) cards with the use of the Vitek Automicrobic System (bioMerieux Vitek, Inc., Hazelwood, Mo.).

D_{10}-values. Each *L. monocytogenes* strain was cultured independently in 100 ml of tryptic soy broth (Difco) in baffled 500-ml Erlenmeyer culture flasks at 37°C (150 rpm) for 18 h. The cultures were then combined and the mixture was sedimented by centrifugation (1,725 × *g* for 30 min). The *L. monocytogenes* cocktail was then resuspended in 40 ml of Butterfield's phosphate buffer (Applied Research Institute, Newtown, Conn.). The *L. monocytogenes* was then inoculated onto the cooked bologna emulsion to a density of approximately 1×10^9 CFU/g and mixed by stomaching for 90 s, and 5-g aliquots were transferred to no. 400 Stomacher bags. For surface-inoculation experiments, the *L. monocytogenes* was applied to the surfaces of frankfurters that had been dipped in SDA solutions. The inoculated sausage was then vacuum packaged to 0.5 mm Hg (ca. 66.7 Pa) with a Multi-Vac A300 Vacuum Packager, overpacked in gas- and moisture-impermeable Mil-B-131-H foil bags (Bell Fibre Products Corp.), and stored at 0 to 2°C until it was ready for use *(18–22)*.

Following irradiation, the samples were assayed for CFU by standard pour plate procedures. Forty-five milliliters of sterile Butterfield's phosphate buffer was added to a no. 400 Stomacher bag that contained a 5 g of inoculated sample or frankfurter, and the sample was mixed by stomaching for 90 s. The samples were then serially diluted in Butterfield's phosphate buffer with 10-fold dilutions, and 1 ml of diluted sample was pour plated on *Listeria*-specific Palcam agar (Difco). Three 1-ml aliquots were plated for each dilution. The plates were then incubated for 48 h at 37°C prior to the enumeration of colonies. D_{10} is defined as the radiation dose required to achieve a 90% reduction in a population of viable microorganisms. The average count (CFU/g) for an irradiated sample (N) was divided by the average count (CFU/g) for the untreated control (N_0) to produce a survivor ratio (N/N_0). D_{10} was determined by calculating the reciprocal of the slope of the $\log_{10}$ (N/N_0) ratio versus the irradiation dose *(18–22)*.

Survival of irradiated *L. monocytogenes* in SDA solution. *L. monocytogenes* was propagated and concentrated as described previously. Five milliliters of *L. monocytogenes* cocktail was combined with an equal volume of 0, 0.25, 0.5, or 1.0% SDA solution (pH 4.5 to 5.0) in sterile borosilicate glass tubes to obtain final SDA concentrations of 0, 0.125, 0.25, and 0.5%, respectively. The samples were then refrigerated (0 to 4°C) for 60 min, irradiated, and pour plated on Palcam medium as previously described.

Gamma irradiation. A Lockheed Georgia Company (Marietta, Ga.) self-contained ^{137}Cs radiation source was used for all exposures. The radiation source consisted of 23 individually sealed source pencils placed in an annular array. The cylindrical sample chamber (22.9 by 63.5 cm) was located central to the array when placed in the operating position. The dose rate was 0.096 kGy/min. The temperature during irradiation was maintained at 4.0 ± 1.0°C by the gas phase of a liquid nitrogen source that was introduced directly into the top of the sample chamber. The temperature was monitored with two thermocouples placed on the sides of the sample bags. The dose delivered was verified with 5-mm alanine pellet dosimeters, which were then measured with a Brucker EMS 104 EPR Analyzer (Brucker, Billerica, Mass.). Radiation doses of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy were used for D_{10} determination. Radiation doses of 0, 1.5, and 3.0 kGy were used for growth promotion and quality factor experiments.

Lipid oxidation. Lipid oxidation was measured by a modified version of the thiobarbituric acid (TBA) assays of Hodges et al. *(7)* and Zipser and Watts *(27)*. Ten grams of bologna was homogenized with 20 ml of 0.5 M phosphate (pH 2.5) buffer containing 0.08% sulfanilamide and 0.01% butylated hydroxytoluene (BHT) with a homogenizer (Virtishear, Virtis, Gardiner, N.Y.) at a speed setting of 70 for 1 min. The homogenate was then centrifuged at 6,700 × *g* for 10 min at 5°C in a Sorvall R2C-B refrigerated centrifuge (DuPont, Inc., Wilmington, Del.) and filtered through Whatman no. 11 filter paper (Whatman, Inc., Clifton, N.J.), and the filtrate was centrifuged at 1,300 × *g* for 10 min at 23°C in a Sorvall RT6000B refrigerated centrifuge (DuPont). A 1.6-ml aliquot of the supernatant was added to a test tube containing 1.6 ml of either −TBA solution (15% [wt/vol] trichloroacetic acid and 0.01% BHT) or +TBA solution (15% [wt/vol] trichloroacetic acid, 0.01% BHT, and 0.65% TBA). Samples were then mixed vigorously, heated at 95°C in a water bath for 25 min, cooled, and centrifuged at 1,300 × *g* for 10 min at 5°C. Absorbance at 440, 532, and 600 nm was measured with a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, Md.). TBA reactive substance (TBARS) values were expressed as malondialdehyde (MDA) equivalents and calculated by the formulas developed by Hodges et al. *(7)*:

$$[(\text{Abs}532_{+\text{TBA}} - \text{Abs}600_{+\text{TBA}}) - (\text{Abs}532_{-\text{TBA}} - \text{Abs}600_{-\text{TBA}})] = A \quad (1)$$

$$[(\text{Abs}440_{+\text{TBA}} - \text{Abs}600_{+\text{TBA}})0.0571] = B \quad (2)$$

$$\text{MDA (nmol/g)} = [(A - B)/157{,}000]10^6 \quad (3)$$

Color analysis. Sausage pieces were packed and irradiated as previously described. Color analysis was then performed with a Hunter Lab Miniscan XE Meter (Hunter Laboratory, Inc., Reston, Va.) *(10, 11)*. The meter was calibrated with the use white and black standard tiles. Illuminate D65, 10° standard observer, and a 2.5-cm port/viewing area were used. Six readings were taken for each parameter.

Shear force. The cutting force for the sausage was measured with a TA-XT2 Texture Analyzer (Texture Technologies Corp. Scarsdale, N.Y.). A TA-7 Warner-Bratzler Blade was used with a test speed of 2.0 mm/s, a distance of 55 mm, and a 20-g autotrigger *(18)*. Maximum shear force was measured 12 times (n = 12) for each treatment.

Statistical analysis. Statistical analysis, including analysis of variance, analysis of covariance, and Duncan's multiple-range test, was performed with Statistical Analysis System software (Version 6.12, SAS Institute, Cary, N.C.). Graphic presentations were obtained with SigmaPlot (Version 5.0, SPSS Science, Chicago, Ill.).

RESULTS

SDA solutions had no affect on the viability of stationary-phase *L. monocytogenes* during the preirradiation refrigerated-storage period. *L. monocytogenes* was more

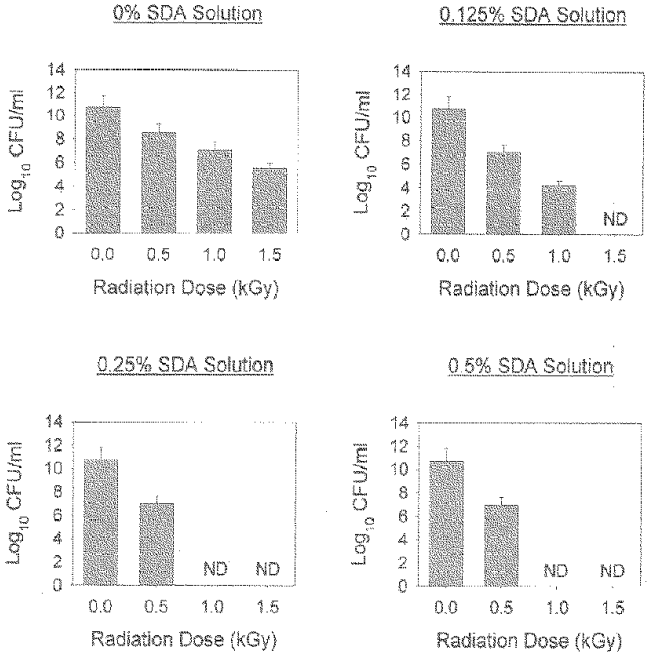

FIGURE 1. *Effect of ionizing radiation and sodium diacetate on the viability of* L. monocytogenes *suspended in solution. Standard error bars are shown for each parameter. Each experiment was conducted independently three times* (n = 3). *ND, not detectable by pour plate assay.*

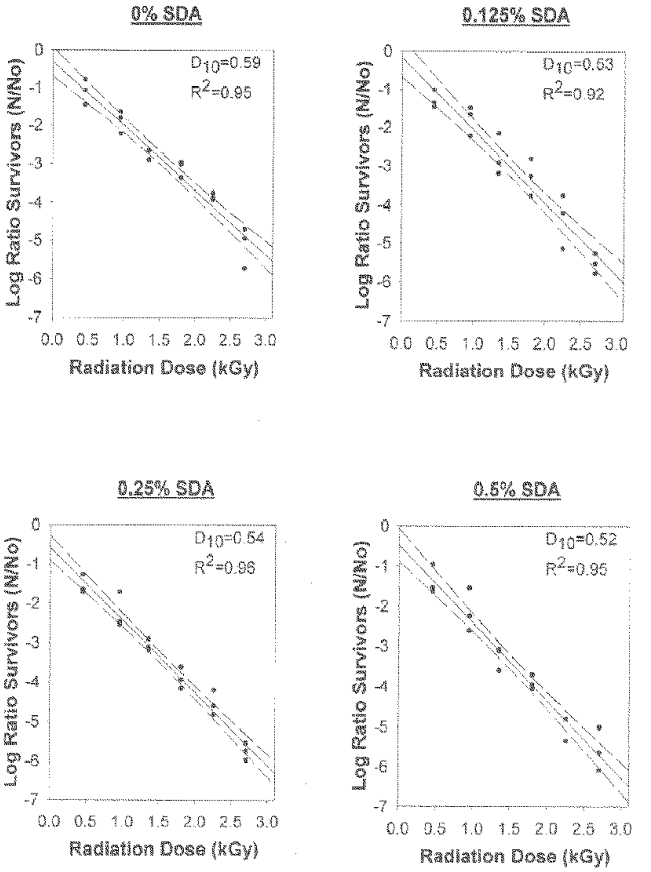

FIGURE 2. *Radiation resistance of* L. monocytogenes *surface inoculated onto beef frankfurters dipped in solutions with various levels of sodium diacetate. The linear regression is shown as a solid line, and 95% confidence intervals are shown as dashed lines. Individual log_{10} reduction values are shown as closed circles. Each experiment was conducted independently three times.*

sensitive to ionizing radiation in the presence of 0.125% SDA solution at doses of 1.0 and 1.5 kGy than to irradiation in the presence of Butterfield's phosphate buffer (Fig. 1). *L. monocytogenes* was undetectable (i.e., a >9-log_{10} reduction was achieved) by pour plate assay following irradiation at 1.0 and 1.5 kGy in the presence of either 0.25 or 0.5% SDA solution. In contrast, irradiation at 1.5 kGy in Butterfield's phosphate buffer without SDA resulted in a 4-log_{10} reduction of *L. monocytogenes* (Fig. 1).

The D_{10}-values for *L. monocytogenes* surface inoculated onto frankfurters dipped in 0, 0.125, 0.25, and 0.5% SDA solutions were 0.59 ± 0.02, 0.53 ± 0.04, 0.54 ± 0.03, and 0.52 ± 0.02 kGy, respectively (Fig. 2). The difference in D_{10}-values was statistically significant for *L. monocytogenes* inoculated onto frankfurters dipped in 0 and 0.5% SDA solutions as determined by analysis of covariance ($n = 3$, $\alpha = 0.05$).

SDA did not affect the viability of *L. monocytogenes* inoculated into bologna compared with the viability of the microorganism in nonirradiated controls as determined by analysis of variance ($n = 3$, $\alpha = 0.10$). D_{10}-values for *L. monocytogenes* were 0.58 ± 0.02, 0.59 ± 0.05, 0.57 ± 0.03, and 0.53 ± 0.02 kGy for bologna containing 0, 0.125, 0.25, and 0.5% SDA, respectively. A comparison of D_{10}-values by analysis of covariance ($n = 3$, $\alpha = 0.10$) revealed a significant difference between the values for the 0 and 0.5% SDA concentrations (Fig. 3).

The growth potentials of nonirradiated and irradiated *L. monocytogenes* suspended in cooked bologna emulsions over 4 weeks of refrigerated storage (9°C) were determined. *L. monocytogenes* cells inoculated into 0% SDA bologna emulsion reached a density >7.8 log_{10} CFU/g after 2 weeks of storage at 9°C. *L. monocytogenes* inoculated into bologna (0% SDA) and then irradiated at 1.5 kGy were able to recover and proliferate without difficulty during the 4-week storage period (Fig. 4). *L. monocytogenes* was detectable but did not proliferate in 0% SDA bologna emulsion irradiated at 3.0 kGy during the 4-week storage period (Fig. 4). However, the proliferation of *L. monocytogenes* irradiated at 3.0 kGy was detected in bologna containing 0% SDA after 6 weeks of storage (data not shown).

L. monocytogenes proliferated, although more slowly, in bologna that contained 0.125 or 0.25% SDA that was not subjected to irradiation (Fig. 4). The recovery and growth of *L. monocytogenes* was slowed but not inhibited by the presence of either 0.125 or 0.25% SDA in bologna that was irradiated at 1.5 kGy (Fig. 4). *L. monocytogenes* growth did not occur in bologna that contained 0.5% SDA and was irradiated at 3.0 kGy (Fig. 4).

The proliferation of *L. monocytogenes* was inhibited by 0.5% SDA, a concentration that exceeds allowable concentrations, regardless of radiation dose (Fig. 4). In order to assess the effect of the treatments on total microbial populations, samples were also plated on tryptic soy agar; however, because of the lack of background microflora, the results obtained were consistent with the results obtained with *Listeria*-specific Palcam medium.

Color analysis indicated small, SDA-dependent, radi-

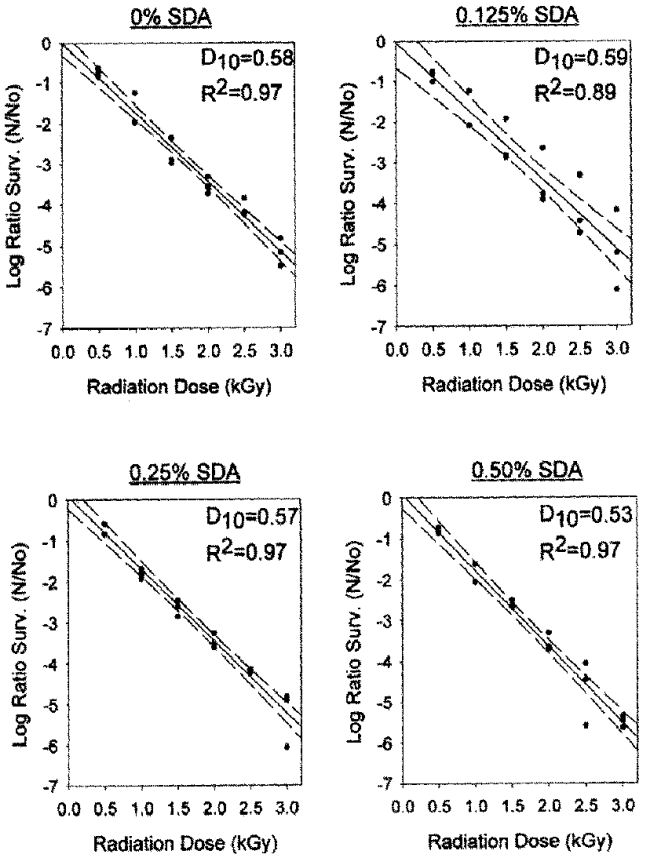

FIGURE 3. *Radiation resistance of* L. monocytogenes *suspended in cooked beef bologna emulsions containing various levels of sodium diacetate. The linear regression is shown as a solid line, and 95% confidence intervals are shown as dashed lines. Individual log_{10} reduction values are shown as closed circles. Each experiment was conducted independently three times.*

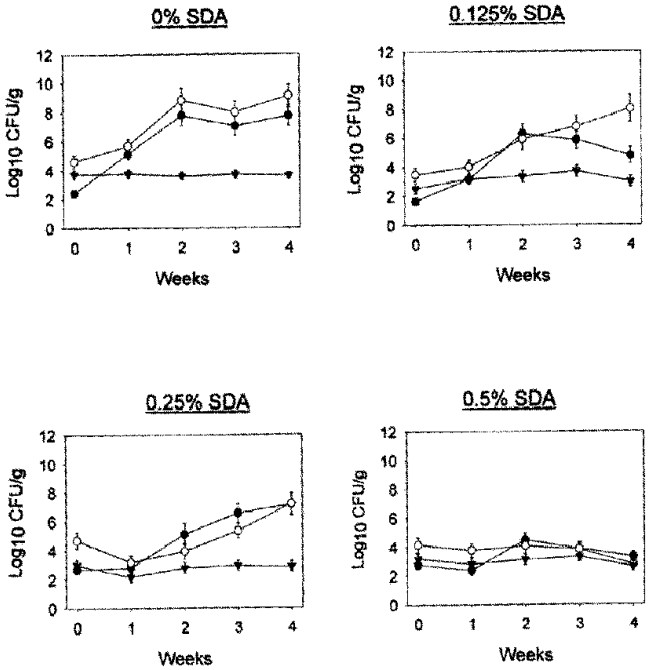

FIGURE 4. *Proliferation of* L. monocytogenes *suspended in cooked beef bologna containing various levels of sodium diacetate over a 4-week refrigerated-storage (9°C) period. Unirradiated samples are shown as closed circles, samples irradiated at 1.5 kGy are shown as open circles, and samples irradiated at 3.0 kGy are shown as triangles. Each experiment was conducted independently three times. Standard errors of the means are shown as bars for each point.*

ation-independent increases in the a (redness) value and the L (brightness) value for unirradiated bologna containing 0.5% SDA relative to unirradiated bologna containing 0% SDA (control), as determined by Duncan's multiple-range test ($n = 5$, $\alpha = 0.05$) (Table 1). A statistically significant radiation-independent decrease in the b (yellowness) value for bologna containing 0.5% SDA was also noted (Table 1).

Lipid oxidation measurements, as determined by the TBARS assay, ranged from 4.16 nmol/g for unirradiated bologna containing 0% SDA to 4.89 nmol/g for bologna containing 0.5% SDA and irradiated at 3.0 kGy. The small

TABLE 1. *Color, lipid oxidation, and firmness of frankfurters made with sodium diacetate and treated with ionizing radiation*

SDA concn (%)	Radiation dose (kGy)	a value (redness)	b value (yellowness)	L value (brightness)	TBARS (nmol of MDA/g of meat)	Shear force (g)
0	0	10.6 ± 0.46 GH	17.2 ± 0.32 A	46.2 ± 0.54 C	4.16 ± 0.12 E	1,734 ± 286 E
	1.5	10.8 ± 0.23 FG	16.6 ± 0.48 AB	47.7 ± 1.02 BC	4.42 ± 0.61 CDE	1,439 ± 275 E
	3.0	9.96 ± 0.59 H	16.2 ± 1.06 BC	47.0 ± 1.04 C	4.29 ± 0.04 ECD	1,646 ± 259 DE
0.125	0	11.1 ± 0.59 EFG	16.0 ± 0.90 BCD	47.5 ± 1.25 BC	4.26 ± 0.20 DE	1,783 ± 133 BCDE
	1.5	11.6 ± 1.02 EFD	14.7 ± 0.47 E	46.2 ± 1.62 C	4.42 ± 0.61 BCDE	1,792 ± 426 BCDE
	3.0	11.2 ± 0.51 EFG	15.9 ± 0.38 BCD	47.9 ± 1.29 BC	4.78 ± 0.12 AB	1,417 ± 398 E
0.25	0	11.7 ± 0.23 ECD	15.5 ± 0.54 CDE	47.6 ± 0.76 BC	4.16 ± 0.10 E	1,730 ± 442 B
	1.5	13.2 ± 1.52 AB	15.3 ± 0.88 CDE	47.8 ± 1.50 BC	4.61 ± 0.07 ABCD	1,977 ± 56.8 ABCD
	3.0	12.3 ± 0.31 BCD	15.8 ± 0.51 BCD	47.2 ± 1.25 C	4.70 ± 0.21 ABC	1,853 ± 538 BCD
0.5	0	12.6 ± 0.57 BC	14.8 ± 0.42 E	49.9 ± 0.95 C	4.28 ± 0.11 CDE	2,090 ± 315 ABC
	1.5	13.7 ± 0.72 A	15.6 ± 1.17 DE	49.0 ± 2.39 AB	4.47 ± 0.10 ABCDE	2,133 ± 540 AB
	3.0	12.4 ± 0.58 BCD	15.0 ± 0.75 DE	49.2 ± 1.40 AB	4.89 ± 0.18 A	2,242 ± 526 A

[a] L (brightness), a (redness), and b (yellowness) values are shown for each parameter tested ($n = 6$). Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) assay ($n = 3$). MDA, malondialdehyde. Firmness was measured by maximum shear force ($n = 12$). Values shown are means ± standard deviations. Means with the same letter in the same column are not significantly different as determined by Duncan's multiple-range test ($\alpha = 0.05$).

but statistically significant increase in lipid oxidation was dependent on radiation dose but not on SDA concentration, as determined by Duncan's multiple-range test ($n = 3$, $\alpha = 0.05$).

Maximum shear forces were equivalent for bolognas containing 0, 0.125, and 0.25% SDA, but bologna containing 0.5% SDA exhibited a radiation-independent increase in maximum shear force. The variation in shear force data can be attributed to the use of a manual sausage stuffer during the manufacturing process.

DISCUSSION

Ionizing radiation can eliminate *L. monocytogenes* from RTE meats, including frankfurters and bologna. A number of factors determine the radiation dose required for a 5-$\log_{10}$ reduction of *L. monocytogenes* in RTE meats. The D_{10}-value for *L. monocytogenes* on RTE meats is dependent on both the formulation of the product and the *L. monocytogenes* strain that contaminates it. Sommers and Thayer *(22)* found D_{10}-values of 0.49 to 0.71 kGy, with a mean value of 0.61 kGy, for *L. monocytogenes* inoculated onto commercial frankfurters. The D_{10}-values for individual *L. monocytogenes* isolates associated with listeriosis outbreaks ranged from 0.46 to 0.62 kGy when these isolates were surface inoculated onto beef frankfurters *(18)*. In this study, the SDA was able to decrease the D_{10}-values for *L. monocytogenes*. Because the ratio of the maximum and minimum radiation absorbed doses (Max/Min) for food products processed at commercial facilities can sometimes exceed 2:1 *(6)*, the identification of additives that increase the efficiency of microbial inactivation is especially important.

SDA has been shown to inhibit the growth of *L. monocytogenes*, regardless of pH, on RTE meat products and may be incorporated into the emulsion at concentrations of up to 0.25% *(16)*. Bedie et al. *(3)* found that SDA slowed the growth of *L. monocytogenes* on vacuum-packaged frankfurters containing 0.25% SDA and completely inhibited growth of the pathogen at 0.5%. Similar results were observed in this study, with 0.5% SDA completely inhibiting the growth of *L. monocytogenes*, while 0.25% SDA slowed the growth of the bacterium. It should also be noted that SDA is bacteriostatic, not bactericidal, to *L. monocytogenes* in RTE meats.

Ionizing radiation is both bacteriostatic and bactericidal to *L. monocytogenes*, depending on the absorbed dose. A radiation dose of 3.0 kGy was sufficient to reduce the *L. monocytogenes* population by 5 $\log_{10}$ CFU/g and to inhibit the growth of survivors for at least 4 weeks following irradiation. A radiation dose of 1.5 kGy eliminated 2.5 $\log_{10}$ CFU of *L. monocytogenes* per g but allowed the recovery and growth of the microorganism. Because Max/Min absorbed dose distributions greater than 2:1 can frequently occur during the irradiation of food *(6)*, the use of SDA, in addition to ionizing radiation, could add an extra layer of insurance for consumers. SDA also has the potential to slow the growth of *L. monocytogenes* and other foodborne pathogens if the product is recontaminated because of accidental package breakage.

Previous work with frankfurters has indicated that irradiation produces little effect on product quality *(24)*. However, no work has been carried out with the use of modern formulations containing antimicrobial additives such as SDA or SDA-lactate mixtures. The lack of major changes to quality attributes including the color, lipid oxidation, and firmness of irradiated bologna containing SDA make the combined use of ionizing radiation and SDA for the prevention of listeriosis a realistic possibility.

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